The Study of Nicotinic Acid Metabolism Using Radioactive Nicotinic Acid, Nicotinamide, and Methionine

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PEI-HSING LIN

B.S. Ginling College, Nanking, China, 1946

M.S. University of Massachusetts, 1949

AN ABSTRACT OF A THESIS

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in the graduate college of the
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THE STUDY OF NICOTINIC ACID METABOLISM USING RADIOACTIVE NICOTINIC ACID, NICOTINAMIDE, AND METHIONINE

The first studies on the fate of nicotinic acid in the body were confined to the estimation of the urinary excretion of free nicotinic acid. It was soon realized that ingested nicotinic acid was also excreted in other forms such as nicotinamide, nicotinuric acid and methylated derivatives. The present investigation has been designed to study nicotinic acid metabolism with the aid of radioactive nicotinic acid, radioactive nicotinamide and radioactive methionine. The technique of paper chromatography was used throughout all the experiments in order to separate and aid in the identification of the known and unknown metabolites arising from from nicotinic acid metabolism.

EXPERIMENTAL

Compounds related to nicotinic acid which were present in urine or tissue extracts were resolved by the following chromatographic solvent systems; 1) the upper layer of a mixture of acetone: n-butanol:H_2O (5:45:50)- ~~referred~~ referred as 'acetone system'; 2) 80% acetone system' plus 20% cellosolve; and 3) 80% 'acetone system' plus 20%morpholine. The locations of the separated compounds on the chromatogram were detected by modified König reaction (1), flurometric (2), bioautographic (3), spectrophotometric and radioautographic procedures.

SUMMARY OF RESULTS

1. It was found possible to resolve a mixture of compounds related to nitotinic acid by paper chromatography. Nicotinic acid, nicotinamide, nicotinuric acid, N'-methylnicotinamide, trigonelline, N'-methyl-2-pyridone-5-carboxylamide, N'-methylnicotinuric acid betaine, nicotinamide riboside, nicotinamide mononucleotide, diphosphopyridine nucleotide (CoI), and triphosphopyridine nucleo- (CoII) were separated in this way. The occurance of some of these compounds as normal metabolites in the urine of different species- human, pig, rat, guinea pig, lamb and calf - was studied.

2. Nicotinic acid and nicotinamide labeled with radiocarbon in the carboxyl position were given by intraperitoneal injection to individual rats. Radioautographs were prepared of paper-strip chromatograms of the urine from each rat. Following nicotinic acid administration the radioautographs showed the presence of 8 metabolic excretion products of which 6 could be identified. The relative amounts of each identifiable product excreted were as follows: CoI 3.5%, N'-methylnicotinamide plus trigonelline 2.7% nicotinuric acid 60.9%, nicotinic acid 24.4%, nicotinamide 0.26%, while one unidentified compound accounted for 7.7% of the activity excreted. Following nicotinamide administration, 10 radioactive excretion products were found on paper chromatograms of which 6 could be identified. The relative amounts of each compound excreted were as follow: CoI 6.08%, N'-methylnicotinamide plus trigonelline 66.9%, nicotinuric acid 0.09%, nicotinic acid 6.6%, nicotinamide 4.1%, and 3 unidentified compounds which accounted for 10.8, 0.84, 0.' 0.99%, respectively, of the activity excreted.

3. A mixture of pure N'-methylnicotinamide and trigonelline could be separated by two dimensional paper chromatography using the " acetone system " followed by the morpholine-' 'acetone system' The two dimensional chromatograms give characteristic patterns of flow for the N'-methylnicotinamide and for the trigonelline. Following the injection of C-14-nicotinic acid or its amide both N'-methyl nicotinamide and trigonelline were present in urine were C-14 labeled and were detected by radioautographs. The occurrance of trigonelline as a metabolite derived from nicotinic acid metabolism was thus demonstrated.

4. Considerable effort has been made to study the nature of the compound (compound 8) res responsible for 7.7% activity excreted from C-14-nicotinic acid or 0.99% activity excreted from C-14-nicotinamide administration. It was found that it could not replace nicotinic acid for the growth of *T. cremoris*. It was inert to CNBr and did not form a fluorescent compound with KOH or morpholine possibly indicating that it does not contain a free ring N and a free alpha position. On acid hydrolysis two products were formed; one promoted the growth of *T. cremoris* and the other reacted with CNBr and with ninhydrin. The behavior of compound 8 toward several solvent systems and its pattern in the

infrared spectrum resembled to that of the 2-pyridone. Compound 8 was also found in the kidney, liver and spleen of rats. It was found to be present in human urine following an oral dose of nicotinamide. Apparently this unidentified compound is an important nicotinic acid metabolite.

5. Evidence for the methylation of nicotinamide could not be obtained with "normal" adult rats given radiomethionine labeled with C-14 at the S-methyl group; but by using severely methyl-group depleted young rats fed a choline free diet plus 1% nicotinamide, transmethylation from radiomethionine to form N'-methylnicotinamide and trigonelline was obtained.

6. There are at least 14 compounds other than N'-methylnicotinamide and trigonelline excreted as radioactive metabolites in the urine of methyl group depleted rat receiving radiomethionine labeled with C-14 at the S-methyl group. Presummably most of these are methylated compounds.

LITERATURE CITED

(1) König, W. J. prakt. Chem. 69, 105, (1904); 70, (1904)
(2) Huff, J. W. and Perlzweig, W. A. J. Biol. Chem. 167, 147, (1947)
(3) Williams, W. L. J. Biol. Chem. 166, 397 (1946)

VITA

Pei-Hsing Lin was born on July 24, 1924, in Tsingtao, China. After graduating from True Light Middle School in Hong Kong in 1942, she entered Ginling College in Nanking, China and received the degree of Bachelor of Sceince in 1946. Before coming to the United States, she worked for one year as a research fellow at the National Institute of Health in Nanking. After graduating from the University of Massachusetts in 1949 with the degree of Master of Science, she was employed by the United States Department of Agriculture in Beltsville, Maryland. She entered the University of Illinois, and has held a research assistantship in the Animal Nutrition Division from September 1949 to July 1952. She was elected into Sigma Xi, Sigma Delta Epsilon, and Iota Sigma Pi, national honorary societies.